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(71) Applicant: Lucas Meyer GmbH & Co., 20539 Hamburg, Germany

Representative: Epping, Hermann & Fischer GbR, 80399 Munich

(72) Inventors:

Schmitt, Heidi, 21521 Aumuehle, Germany

Michael, Dr. 21244 Rosengarten, Germany

Michael 21441 Garstedt, Germany

(56) Citations:

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The following information has been taken from documents submitted by the applicant:

The test application was submitted in accordance with § 44 of the German Patent Law (PatG).

(54) Method for Manufacture of Phosphatidyl Serine (PS)

(57) The invention relates to a method for manufacturing phosphatidyl serine (PS) or of PS-enriched products in which Lecithin is dispersed in water and D-, L- or DL series are added. Phospholipase C and $CaCl_2$ are dissolved in water and transferred to the dispersion. After 10 to 20 hours of stirring at room temperature, the calcium salt of the PS is separated from the aqueous phase, the free L-serine and choline are washed out. Finally by means of ethanol extraction, PS or PS-enriched products are obtained which exhibit no residual enzyme activity.

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1 Description

Known methods for producing PS are based on the following characteristics:

- Reactions in two-phase systems
- Use of toluol, MIBK, diethyl ether or ethyl acetate as solvents, and water, and
- Use of large L-serine surpluses.

Due to the presence of residual solvent, instability of formulations and costs, these methods are only partially suited to the manufacture of products used in food additives and in so-called 'functional foods'.

The advantages of the method in accordance with the invention are:

- No solvents, since the reaction takes place in a single-phase system using water as solvent
- The use of conventional raw materials, namely phospholipase, D, L-serine, calcium chloride, phospholipids (lecithins).
- The use of a substrate in its natural composition(s), namely lecithin (e.g. from soy, eggs) up to purified phosphatidyl choline (PC).
- The use of a small amount of L-serine , namely a dosage applying to substrate in the range of 0.2:1 to .2:1, and thereby a clear reduction of the excess from max.50:1 to 0.2:1 whereby the theoretically required amount is 0.14:1.

Further advantages of the method are:

- High selectivity for converting PC into PS and

- Few adverse reactions, e.g. hydrolysis of PC into PA, occur.

In accordance with the invention the recommended procedure is as follows:

Lecithin is dispersed in water (1-20%, preferably 5%) and added to the L-serine solution. The phospholipases D and $CaCl_2$ are dissolved in a small amount of the water and subsequently transferred quantitatively to the dispersion. The dispersion is stirred over a period of (preferably) 10 – 20 h, preferably at RT.

After the reaction has ended, the resulting calcium salt of the phosphatidyl serine is separated by conventional methods from the aqueous phase, since it is insoluble in water.

The free L-serine and choline in the product can be removed by a further washing with water.

The product can then be dried. e.g. by freeze drying or spray drying. Residual enzyme activity can be combated by a deactivation, e.g. subsequent ethanol extraction of the reaction product

In doing, so, one simultaneously achieves PS-enriched products that are stable and do not demonstrate any residual enzyme activity. The conversion of Ca salts into free PS or PS sodium salt, for example, can be performed by any known method.

By embedding or including PS and PS-containing products in a solid fat, which is stable at room temperature, the invention enables the development of PS products with stable formulations in aqueous systems. This is because the product is protected against hydrolytic reactions (e.g. application for beverages containing fat-encapsulated without the possibility of the PS being hydrolyzed. For the enclosure of soft gelatin capsules, the

melting point of the fat is selected in such a way that it is minimally below the encapsulation temperature.

Since the phosphatidyl serine is enclosed in a fat inside the capsule, which does not melt at room temperature, but is nonetheless liquid at body temperature, i.e. below 37^0, it ensures that the water content of the gelatin mass does not affect the enclosed PS. The advantage is that absolutely no hydrolysis occurs then. A melting point can be set that is low enough to enable good absorption of PS in the gastro-intestinal system –at body temperatures at any rate.

In accordance with the invention, this principle can also be applied to other substances that can be attacked by water in a soft gelatin capsule.

Example for a Solid Fat

SATINA 15 is a lauric plant fat that is fractionated, hardened and refined.

Characteristic Values

Iodine value (Wijs): 7g/100g

Free fatty acids (laurinic acid): 0.03%

Peroxide number: 0.1 meq02/kg

Trans fatty acids (approx.), 1%

Lovibond color (5.25" cell)

Yellow: 4.2

Red: 0.7

Solid fat share at

20°C: 71%

25°C: 42%

30°C:1 35 °C

Slip melting point: 29°C

L-Serin Properties

(S)-2-amino-3-hydroxy-propanoic acid

L-2-amino-3-hydroxy-propionic acid

$C_3H_2NO_3$

M=105.09 g

White crystals or crystalline powder

Practically odorless

Solubility; 35.9 g/100 g H_2) 20°C

Patent Claims

A method for the manufacture of phosphatidyl serine (PS) or of PS-enriched products, in which lecithin is dispersed in water (1-20%) and L-serine is added, Phospholipase D and Ca Cl_2 are dissolved in water, quantitatively transferred into the dispersion and stirred for about 10-20 h at room temperature. The PS calcium salt that is insoluble in water is separated, the freed L-serine and choline are removed by a extraction with water, and after ethanol extraction, PS or PS-enriched products (is/)are achieved.